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VARIATION AND HEREDITY IN LUPINUS

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THE great majority of genetic investigations have been carried on with domesticated plants that have been long under cultivation. There are, of course, many entirely good reasons for this fact. It has, however, always seemed to the author that the principles of genetics might, perhaps, be more successfully approached by beginning with wild plants. For this reason he has been bringing into the garden during the past several years a considerable number of wild species which appeared to offer desirable material for experimentation.

His attention was first directed to *Lupinus* through the difficulties that appeared to beset the systematist in arriving at a satisfactory classification of the species. It seemed possible that the difficulty might be due to the fact that the species are of recent origin and, so, very close together. Under the circumstances that a genus is either now or has recently been in a mutable state it would seem likely that natural selection would not yet have had time to weed out all the forms doomed to perish through lack of adaptation or insufficient vigor.

While discussing this question one of his colleagues mentioned the fact that the form recently (1911) described under the name of *Lupinus piperSmithii* Heller (5) grows near and that it sometimes has pink flowers. An investigation during the spring of 1914 not only confirmed this statement, but also showed that pink flowers are not uncommon in two closely related species—*L. vallicola apricus* (Greene) (4) and *L. nanus* Dougl. (7). These three species are all close to one another but apparently satisfactorily separable by the systematist.

This will be clear from the parallel descriptions given below. That each of the species may produce variant types with some characters of the others will appear in our further discussion.

<i>L. piperis</i> <i>smithii</i> Heller	<i>L. vallicola</i> <i>apricus</i> (Greene)	<i>L. nanus</i> Dougl.
Annual	Annual	Annual
Somewhat pubescent below with white hairs, densely so above, especially so in the inflorescences.	Rather shortly and sparsely pilose.	Villous or finely pubescent.
Branches several from the base, 40 cm. high, straw colored, rather prominently ridged.	Branches few to several, almost upright, firm, from near the base, 30 to 45 cm. high.	Slender, not succulent, often branching from near the base, 15 to 37 cm. high.
Petioles slender, about 5 cm. long or uppermost somewhat shorter.		Petioles $\frac{1}{2}$ to 3 times longer than leaflets.
Leaflets about 8.2 cm. long, 2 mm. wide above gradually tapering to the base, apex slightly narrowed and acutish, sparingly appressed pubescent above, more so beneath, light green in color.	Leaflets about 7, oblong-linear, acute, about 1.5 cm. long, thin, sparsely appressed pubescent.	Leaflets 7 or 8, linear to oblanceolate, 1.2 to 2.5 cm. long, usually acute.
Stipules subulate, green, 6-7 mm. long.		
Peduncles 5 cm. long, or less, the inflorescence about the same length.	Racemes sub-sessile, 5 to 8.6 cm. long in full flower.	Raceme loose, short peduncled, 7.5 to 17.5 cm. long.
Flowers in 3 or 4 whorls, the internodes except the uppermost longer than the flowers.	Flowers in 4 or 5 verticils, these rarely indistinct.	Flowers fragrant and in several distinct or somewhat indistinct whorls.
Bracts subulate, 4 mm. long, caducous.		Bracts exceeding the calyx, deciduous.
Pedicels 4 mm. long, slender, villous with short white hairs.		Pedicels about 6 mm. long.
Calyx white villous but the green showing underneath; upper lip long, parted for nearly 3 mm., the lobes lanceolate with a broad V-shaped sinus, the whole broadly ovate when spread out, 4 mm. wide at base; the lower lip oblong, 5 mm. long, 2 mm. wide, 3-toothed at the apex.	Calyx—broad upper lip deeply cleft, the lower entire, the whole exterior appressed villous but not densely so.	Upper lip deeply cleft, lower 3-dentate, the middle tooth sometimes obscure or wanting.

Corolla bright violet blue, 7 mm. long, 5 mm. deep, distance between apices of wings and banner less than 2 mm.

Banner appearing as if shorter than the wings, the edges turned back and slightly crinkled above, obovate-cuneate when spread out, 6 mm. wide, with a shallow median groove turning purple or violet in age.

Wings inflated, open dorsally and at the apex exposing the keel, the ventral edge closed, raised into a low sharp keel, the individual wings obovate-oblong when spread out, 5 mm. wide just above the middle, then obliquely rounded to the broad blunt apex.

Keel glabrous, moderately curved, 2 mm. deep across the middle, the apex purple.

Pods yellowish, villous, 2.5 cm. long, 4 mm. wide, about 7-seeded.

Seeds pale flesh-colored, slightly yellow-brown mottled, 2 mm. long, 1.5 mm. wide.

Corolla (blue) broader than long, the breadth about 10 mm.

Banner with white middle spot, purple dotted along the median sulcus, turning reddish purple in age.

Banner with white middle spot with purple dotted sulcus turning reddish purple in age, orbicular retuse with the sides reflexed.

Wings lightly joined, forming an obliquely ovate, inflated sac.

Keel naked (Greene) or bearded on the apical half (Smith).

Pod 2.5 cm. long, appressed villous, 3 to 7-seeded.

Seeds not strongly compressed, obliquely round-oval, grayish with a few markings and many small dots (Greene).

Seeds well marked with prominent dark brown line on each side and rest of the surface thickly marbled or finely dotted with dark grayish brown. (Smith)

The above descriptions have been taken from the original sources for *L. piper Smithii* (5) and *L. vallicola apricus* (4) and from Jepson's Flora of Middle Western California for *L. nanus*. The three species are illustrated in the accompanying text-figures.

Field Study of Variation.—Field work can be carried on profitably only in years when lupines are abundant. 1914 was a very favorable year for *L. vallicola apricus* and 1919 and 1920 for *L. nanus*.

In 1914 *apricus* and *pipersmithii* were very numerous in a field near the university and much study was devoted to them. Besides the pink forms already mentioned a considerable number of other color variants were noted and seed collected of some of them. Seeds were collected of both the pink and blue forms of *pipersmithii* and have since been cultivated. Smith (10) had earlier collected seeds of these varieties, but had not been successful in cultivating them. Jepson (7) makes note of the variabil-

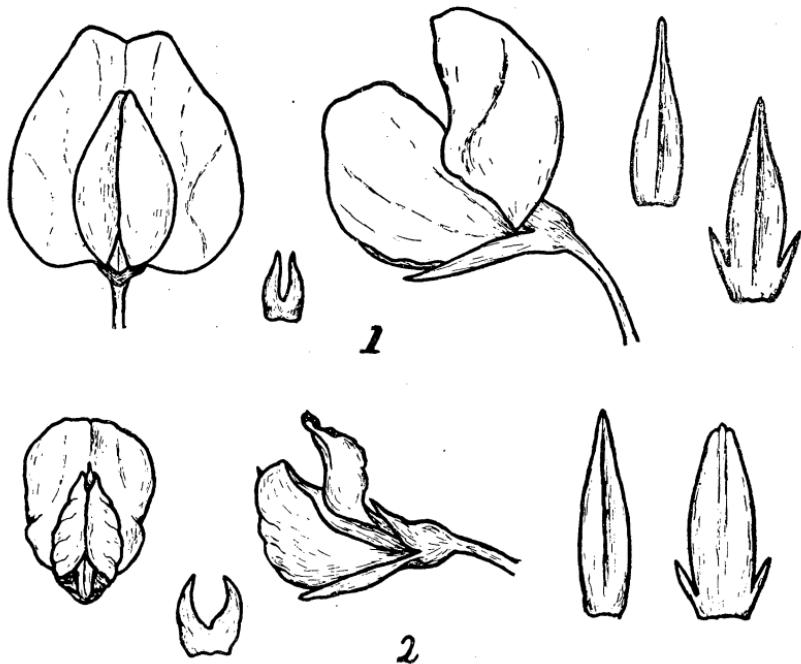


FIG. 1. *Lupinus vallicola apricus*, showing the flower and its parts.
(After C. V. P. Smith.)

ity of *nanus* and Smith mentions variations of size of plant and flower in *apricus*.

The normal color of *apricus* is dark blue and white. After some study it became very obvious that, mixed with the usual types, there were occasional plants with a decidedly lighter blue. One variation consists in a mere lightening of the blue. This type was called light blue

and plants were bagged for seed collection. Another type resembled this in color except that both banner and wings were distinctly striped. The amount of blue and white varies somewhat but the type is always recognizable. In some cases one might correctly speak of a light blue flower

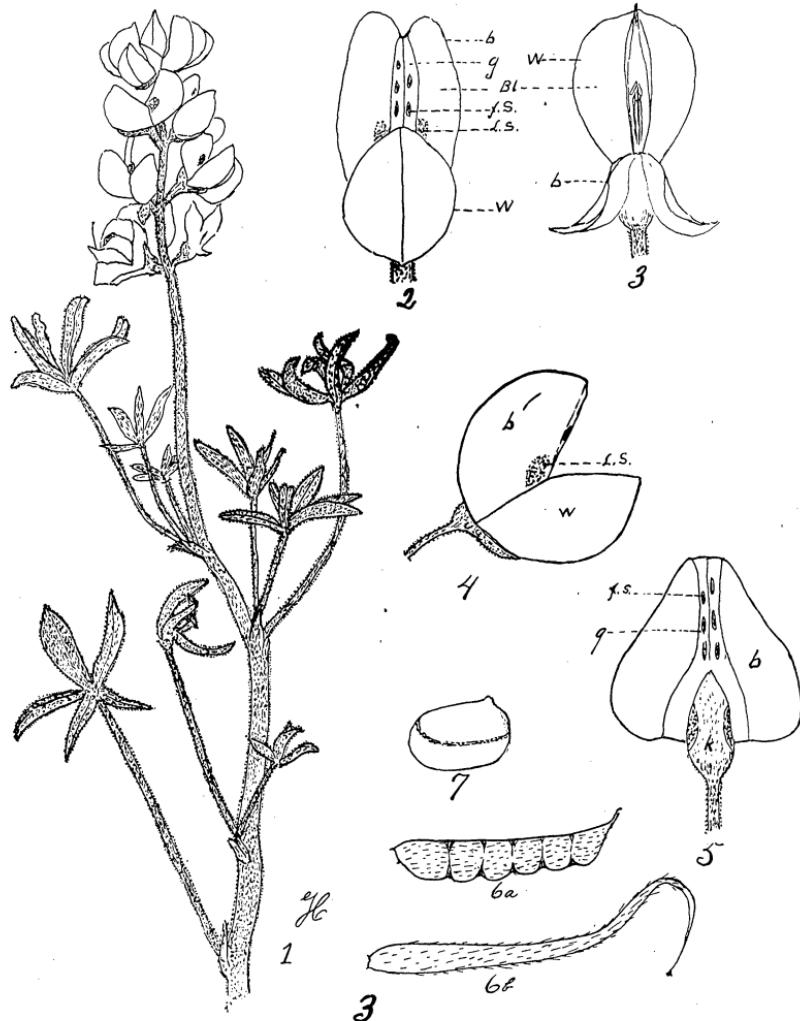


FIG. 2. *Lupinus piper-smithii*, showing floral parts. (After C. V. P. Smith.)

FIG. 3. *Lupinus nanus*. 1. Normal plant. 2. Single flower, front view. 3. Looking down on flower showing particularly the banner shape. 4. Side view of single flower, showing position of lateral spot. 5. Front view with wings removed showing position of frontal groove and spots. 6a. Pod of normal plant. 6b. Pod from "curly pod," type 11 in table. 7. Seed from Pedigree V, showing dark ring.

with white stripes or veining. In other, and rarer, cases it is apparently a white flower veined with blue. Seeds of this type were collected and have since been grown under the name of striped white. This first season it was not realized that the two lighter forms were distinct. Fortunately the seeds of each individual plant were saved and grown separately, so that the distinction became evident in 1917, the first year in which successful cultures were grown.

Pink *apricus* were also fairly common that year and have been occasionally noted since. Seeds were saved and have since been grown.

Two white forms were observed that year. One appeared to be plain white. This type has been frequently observed since in this and other species. A single plant was also observed in which the color was a cream or yellowish white. Seeds of both sorts of plants were saved but have so far failed to grow.

A few scattering observations were made on *nanus* from year to year, but it was not until 1917 that a field was found in which variations were numerous and conspicuous. Pinks, light blues, striped whites, and whites were all abundant in a field near the town of Woodside. Specimens of all these types were staked out for seed but unfortunately attracted the attention of the small boy and no seeds were secured. In 1919 3 fields were found some 3 miles west of the university in which numerous variations were in full bloom. A careful study was made and an attempt made to discover and describe every variation. Twenty-seven plants were labeled and described for seed collection. They represented 12 separate and distinct variations or combinations. All the variations but two are concerned with flower color. In the description of *nanus* it will be noted that the normal plant has a blue and white corolla having a reflexed banner with a white front down the middle of which there is a groove marked with a number of small dark spots. In the variants the normal dark blue of the banner and

wings may be replaced by the light blue or striped white described for *apricus* or the whole corolla may be white or pink and white.

All the dark blue, light blue and striped white plants had a white front on the banner with blue, blue-green, or no spots in the groove. White corollas had either white or lemon-yellow fronts with or without the dark spots. Normal dark blue plants have the color uniform on banner and wings or else with a deeper spot at the angle where the reflexed banner joins the wings. In light blues this spot may be dark blue, light blue, greenish blue or absent altogether. Whites have this spot faintly blue, bright orange, or absent. The orange spots may occur with either white or lemon-yellow fronts.

A reddish-purple corolla was found on a number of plants which were distinguished by unusually large and fine flower clusters. In some of them the flowers had fallen and pods formed on some earlier branches. The pods in every case were of a very peculiar type. Instead of the elongated, nearly straight and somewhat flattened and constricted type commonly found these plants had pods long, tapering, nearly round, and frequently much coiled. This was at first supposed to be due to attacks of insects or fungi. Diligent search failed, however, to show any such infection or infestation. And when it was later discovered that the plants which would produce these curly pods could be identified in the early flowering stage it was realized that this whole group of characters constituted an interesting variation. A number of plants were labelled and some bagged for seed. Although many seed capsules were examined, no good seed were secured. It appears highly probable that this form is completely sterile, though there is a chance that the failure to set seed may have been due to unfavorable weather. In 1920 neither this nor any of the other variations were to be found in that same field. Diligent search, in fact, has failed to discover it anywhere. It is much to be regretted that good seed of this

form can not be secured, inasmuch as the somatic variations involved appear to be more profound and complicated than in any of those so far studied.

The only other form variations, aside from mere size, so far observed are two modifications of the banner. In one plant of *nanus* in 1919 the banner was hooded in the fashion of some varieties of sweet peas. This same variation had been previously noted in *apricus* in 1914. *Apricus* also occasionally yields plants with the abbreviated banner characteristic of *pipersmithii*. So far seeds of neither of these variations have been collected, though attempts have been made.

The table below summarizes the variations observed in 1919. This season's study yielded no new types and failed of some of those found the year before.

Number	Color and Markings		Front	Front	Lateral	Other Characters
	Corolla	Veining		Spots	Spots	
1.....	Light blue	Veined	White	Dark blue
2.....	"	"	"	Dark blue	Dwarf
3.....	"	"	"	Faint blue
4.....	"	"	Greenish	Greenish
5.....	White	{ Lemon-yellow }	Faint blue
6.....	"
7.....	"		Dark blue	Orange
8.....	"	Lemon-yellow	"
9.....	Pink	White	Faint blue	Dwarf size
10.....	"	"	Dark blue	Blue
11.....	Reddish purple	"	"	Curly pods
12.....	Reddish purple	"	"	Blue	Hooded banner

The variations in size of all three species are very considerable, no doubt due in large part in the field to differences of soil, moisture, shade and exposure. When grown in the garden side by side *nanus* is the largest and most vigorous and *pipersmithii* the smallest. In the field *nanus* is often smaller than *apricus*, though the flower cluster is usually much larger. This difference is possibly due to the fact that it grows in more open, ex-

posed places and usually in poorer soil. No cultures of different-sized plants of the same species have yet been grown and critically compared. Some casual observations, however, indicate that there are probably heritable size differences in *apricus*.

Reference to the specific descriptions will show that some taxonomists have been inclined to base classification in part on the color, shape and markings of the seeds. Our observations would indicate that this is not a very safe criterion, particularly in reference to color and markings. Seeds of *nanus* are the largest. They could usually be picked out of a mixture with *apricus* by size and shape, but not by the color or markings. No character is more subject to variation than this. This does not mean that the character is a fluctuating one, but that there are a great many differently colored and marked seed varieties. All the seeds of any one plant are alike (with certain exceptions to be noted in a later paragraph), as one would anticipate since the color and markings are seed-coat characters and hence genetically all alike on any particular plant.

Dark blue *nanus* and *apricus* plants have dark seeds, whites and pinks have light seeds, light blues and striped whites have seeds of intermediate color. Pinks and light blues have a yellowish-brown tone and are flattened and without other markings. Striped whites are longer and thicker in proportion to the width, of either a bluish or yellowish tone with a conspicuous crooked line on either side forming a continuous ring about the shorter perimeter of the seed. This ring does not occur on the seeds of pinks or whites. It is present on some races of dark blues and absent on others. The statements just made apply particularly to those races which have been cultivated for some time in the experiment garden. Field observation confirms them in part, but reveals a considerable number of other variations in color and marking which have not yet been cultivated and about the genetic behavior of which nothing is yet known. The only case

so far noted in which the seeds of a single plant of *apricus* are not entirely uniform is that of two tones in a striped white variety. An attempt has been made to cultivate the two sorts separately and to determine the ratio in which they occur. Cultures have not yet proved successful. In some collections of seed the ratio approximates a 3:1, but in others it is nearer 8:1, but whether any significance is to be attached to these results has not yet been definitely determined.

It is a curious fact that, although lupines yield an enormous number of seeds and the plants often literally cover acres of space they are nevertheless very capricious in their occurrence. Some illustrations of this may be taken at random from our notes. The field near the university where the *apricus* mutants and the pink *piper-smithii* were collected in 1914 has been under observation every year since and has not at any time had many plants or produced a second display of these mutants. A number of pink forms of both species were staked for seed that first year and the stakes left in the ground to mark the site, in the expectation that the same forms would reappear the following year. In no single case were pink flowers found at any of these stations, although they were found the next year at other locations in the same field. The field near Woodside studied in 1917 has been visited each year since. Not only have no mutant forms been found there, but there have been exceedingly few normal ones. The field from which the notes were made for the table on a preceding page was very thoroughly searched again this spring. There were a few dark blue plants, but not a single one of the types which were more or less abundant there last year. These vagaries of distribution doubtless depend in some manner not yet clear on the difficulty of germinating the seeds.

Pollination and Seed Collection.—In 1914 when these observations were begun it was assumed that the lupines were probably frequently cross-pollinated, inasmuch as they appeared to be freely visited by bees. It was a

matter of some surprise to find that the forms of *apricus* and *pipersmithii* which were brought into cultivation did not indicate this to be true. The pinks bred true in both species. Experiments to determine self-pollination by bagging or screening the plants with fine-meshed wire cages showed no diminution in the harvest. Furthermore, in the following years different strains grown in adjacent rows showed no sign of crossing. It was then assumed that the same would be true of *nanus* and flower clusters were bagged in the field for seeds—not to insure selfing, but merely to prevent the seeds from being scattered by the explosive dehiscence of the pods. The results were wholly negative, resulting in a failure to secure any seed that year. The appearance of the contents of the paper bags first used led to the supposition that possibly the failure to set seed was due to the bags. Careful experiments were therefore made the following season by inclosing whole plants in cages of fine screen wire or cheesecloth. In no case did this result in setting seed. It appears, therefore, that *nanus* is dependent on bees for pollination. On the other hand, cultures derived from white-flowered *nanus* have shown that the bees act in part merely as a mechanical agent, for part of the progeny was white and part blue. These results agree well with and serve to explain the greater number of variations found in *nanus*.

Pollen Sterility.—It has been maintained by a number of authors at one time or another that variability in nature is very largely a matter of hybridity and that sterile pollen is a more or less certain indication of the hybrid nature of a species (6). Having found two closely related species both variable and in a closely similar manner, it became a matter of interest to study the comparative sterility of close-pollinated and cross-pollinated species. In order to determine this matter a large number of plants belonging to all the varieties in cultivation at the time in the garden were examined. Mounts of pollen from three different flowers from each plant were

made. Each slide was so prepared that about 100 pollen grains would be visible in a single field of the microscope. All the grains in the field were then counted and the percentage of sterile grains calculated and averaged. Only two plants showed more than 3½ per cent. of sterile pollen. One normal dark blue plant showed 40 per cent., 39 per cent. and 0 per cent. in three flowers. One striped white showed 9 per cent., 6 per cent. and 0 per cent. in three counts. One hybrid light blue, one dark blue and one pink showed no infertile pollen. The one plant with a high degree of sterility was a selfed dark blue *apricus*. Only a few plants of *nanus* were available, but they showed no poorer pollen than *apricus* and *pipersmithii*.

Seed Germination.—In a previous paragraph it has been pointed out that, although lupines produce immense quantities of seeds, field germination is apparently poor—at least an abundant seed harvest is likely to be followed by a poor stand of plants the following season. Our earlier attempts to grow them in the garden were practically a total failure.

The first attempt to grow controlled cultures was made in the winter and spring of 1914-'15. Four hundred seeds were planted in pots in ordinary unsterilized garden soil and 400 more in pots of sterilized soil (sterilized in an Arnold steam sterilizer). The seeds themselves were planted dry without treatment of any sort. The results were almost a total failure. About 5 per cent. of the seeds produced seedlings. Of these all but 5 or 6 were killed as seedlings by the attacks of soil fungi or just simply died. Two plants lived to flower, but failed to set seed. It appeared evident that greenhouse cultures under the conditions then available were likely to be unprofitable and the remainder of the original seed collections were held over until an experiment garden could be secured.

In 1917 the remaining seeds were planted without treatment in the open garden. Nine plants in all lived to mature seed—one light blue, one striped white, one pink

pipersmithii, three pink *apricus*, and three dark blues. The seeds since used have all been derived from these nine plants, each of which has been assigned a pedigree number.

In 1918 seeds of each of the 1917 plants were again planted. Although about 400 seeds of each were planted in each culture, two pedigrees failed to yield a single plant that year, though a few plants came up the following spring. Pedigree VIII, Light Blue, yielded a culture of 85 plants and pedigree V, Striped White, 28 plants. Thus the highest per cent. of germination did not exceed about 20 per cent., ranging from that down to zero.

In 1919 a series of germination tests were carried out. This had not been possible before on account of the small number of seeds available. A considerable variety of methods were employed. Soaking the seeds in tap and distilled water for weeks is of little value, owing to the failure of the seed to imbibe water. Breaking or cutting the seed coat brings about prompt imbibition of water and consequent swelling, but does not produce a high percentage of germination. Seeds were soaked in water under air pressures up to 140 pounds to the square inch with no noticeable effect.

Since the seeds are small cutting or filing the seed coats is a very arduous affair where cultures of any size are to be grown. Attempts were, therefore, made to find some other means of bringing about the same result. The seed coat appears to be difficult to wet and this was thought to be due to the presence of some oily or waxy constituent. Attempts to dissolve this by means of KOH, various percentages of alcohol, ether, etc., proved entirely unsuccessful.

Soaking in concentrated H_2SO_4 proved the most efficacious method tried. This was applied in parallel series of seeds from the same plant for periods from 5 minutes up to 2 hours. The shorter treatments seemed to produce no effect at all. The longer periods of two hours or over killed the embryos. After some experimentation it

was found that a treatment of one and one half hours would sufficiently char the seed coat to secure practically 100 per cent. of swelling (8). At first it seemed impossible to say whether this might not also injure the embryos. Later it was discovered that whenever any acid penetrated the cotyledons of dark blue plants they turned pink. This color is probably due to the presence of a chromogen similar to or identical with the one which eventually produces the pink or blue flower pigments. Since this color reaction is brought about by even faintly acid solutions, it was thought that it would serve as an effective check against overtreatment with the acid.

After the preliminary experiments had shown the sulphuric acid method to be the best at our disposal a complete series of tests were run on each of the 70 pedigrees available for planting at that time. The dry seeds were placed in the concentrated commercial acid and left for 90 minutes. They were then washed rapidly through several changes of sterile water until the water failed to affect litmus paper after the seeds had stood in it for 20 to 30 minutes. It was found necessary to carry out the washing rapidly since the weak acid readily penetrated into the cotyledons.

After washing the seeds were subject to one of three treatments. Some were left in sterile water until sprouted. Others were removed as soon as they had swelled. The great majority of the pedigrees swelled within 18 hours. A few were completely swelled within 4 hours. In most cases they were left about 18 hours. A third method was to transfer the seeds as soon as washed to a nutrient solution. This did not show any advantage over plain sterilized water. The seeds which were removed from the water after washing and swelling were placed on or between moist blotting papers. It was soon found that those left in the water or placed between papers kept wet and soggy excelled those which were placed on papers merely kept moistened. Twenty-one lots of seed failed to sprout at all, although the percent-

age of swelling was 95 per cent. or better except in two cases, each of which had only 40 per cent. of the seeds swelled. The remaining lots averaged 51 per cent. of sprouted seeds. Two gave 100 per cent., one gave 95 per cent. and 14 were below 25 per cent.

From these results it appears that many seeds which show no observable defects are nevertheless either dead or in a state of dormancy not readily overcome. That the latter is probably the true explanation is indicated by the fact that more seeds treated in this manner actually sprout than when the seed coats are mechanically ruptured. The treatment with the acid seems to act in some manner, possibly by dehydration, as a slight stimulant to sprouting. It is not certain, however, that a larger percentage of viable seedlings is actually produced. Many seeds put forth the radicle in an apparently normal manner, but do not continue growth. Others die at later seedling stages apparently from internal causes, for they have not had opportunity of infection and are growing under the same conditions as others in the same culture.

This spring and winter a number of cultures were tried in which the seed were treated with acid, washed, and then planted directly in the soil out of doors. In every case a good stand was secured averaging about 50 per cent. of the seeds planted. The seedlings of the preceding season were planted in pots and kept in the greenhouse until a vigorous young plant was secured, and then transplanted to the garden. They did very poorly after being transplanted and produced practically no seed. It is uncertain whether the failure was due to faulty technique in transplanting or to the failure of the plant to adapt itself to the change of environment. It is not unlikely that both causes had something to do with the matter. They had been grown in 5-in. pots and transplanted with the whole mass of dirt, but even in that way some disturbance of the root system was unavoidable. In addition to this there was a very hot, dry wind lasting three days about flowering time. This seemed to do a lot of

damage to plants in the field and was no doubt highly injurious to those in the garden as well. It is planned to repeat the experiment again, using paper pots which may be set in the garden without disturbing the roots at all and without interfering with subsequent growth. (These pots have no bottoms.)

Garden Cultures have now been carried through four seasons in some lines and through two or three in the others. Pink *apricus* and *piperSmithii* have proved entirely constant, with one exception. Dark blue *apricus* also breeds true.

The striped whites of Pedigree V for three seasons produced both striped whites and dark blues. In 1918 the culture produced 28 plants which flowered. There were 20 striped whites and 8 dark blues. In 1919 the cultures were so badly injured by transplanting and unfavorable weather that little reliance could be placed on numbers. Some cultures, however, did produce white plants. In the larger, but still unsatisfactory, ones this spring whites have again been produced. Although the numbers are very small, the fact that striped whites give rise to dark blues, striped whites, and some whites is significant. Two cultures of 100 seeds each this spring produced two white plants each and no other sorts. From these data, unsatisfactory as they are, it is probable that striped whites are hybrids between white and dark blue and that white differs from dark blue by a single factor.

The light blues of Pedigree VIII have also been grown through four seasons. In 1918 the original plant produced a progeny of 85 plants, of which 20 were dark blue, 60 light blue, and 5 failed to flower. They have never produced any whites. This season 8 cultures out of 27 produced only light-blue plants. In most cases the numbers were small, but one culture of 200 seeds produced 32 light blues and one plant which did not flower. Three others respectively produced 22 light blues out of 200 seeds, 18 light blues out of 170 seeds, and 9 light

blues out of 150 seeds. Taking into account that four of the eight cultures had only one or two plants and so might have produced dark blues also, this 8 : 27 is probably as close an approximation to a 3 : 1 ratio as could be expected. The data now available would appear to justify the conclusion that the light-blue color is due to a single factor difference and that heterozygotes and homozygotes are phenotypically indistinguishable.

Owing to the fact that a single cross yields only five or six seeds, it has not seemed profitable to attempt hybridizing the light blues and striped whites either with one another or with other forms, until a technique has been perfected that insures a higher percentage of germination. In *nanus*, which is naturally crossed, certain observations have been made which indicate something of the relations of certain factors to one another. Seeds of a white-flowered plant collected last year from unprotected flowers were grown this year and produced both whites and blues apparently like normal wild ones. This would indicate that the mother plant had been visited by a bee which had effected pollination in part with its own pollen and in part with that of a neighboring dark blue. If this be the true explanation this white was a recessive one. It might have been a heterozygous dominant white, of course, which would be in agreement with the nature of the whites in *apricus*, Pedigree V. Seeds of pink *nanus* collected last year yielded only a half dozen plants, all dark blue. This would point to the conclusion that pink is also recessive to blue. However, these results are too meager to have more than a suggestive value.

Seed characters have also proved constant in inheritance. Dark-blue *apricus* plants invariably have dark seeds, but the particular type of marking differs according to the origin. It is suspected that the two types of seeds found in Pedigree V, striped whites, indicate a genetic difference, possibly distinguish striped whites

from pure whites, but the facts now known do not suffice to prove this.

Mutations have apparently occurred in culture in respect to both flower color and seed-coat markings.

This spring (1920) a single dark blue appeared in a culture of pink *apricus* which had bred true through the three preceding generations. In 1918 a pink arose in a culture of seeds from a wild dark-blue *nanus*. If the relations between pink and dark blue are the same in the two species one of these cases must be a mutation. Hybridization is exceedingly improbable in the case of the pink *apricus*. It is not likely that the dark-blue *nanus* was a hybrid either since it was not collected from a location where this cross would have been likely to occur and only one plant out of a large culture was pink.

In 1918 plant VIII-27 with dark-blue flowers produced seed of the light color characteristic of light blues. These seeds this year produced both light blues and dark blues. In collecting these seeds it was necessary to read the label on the plant and that on the seed box. It is very unlikely that they failed to tally with each other or with the color of the flowers still in bloom on the plant. Several collections were made over a period of two or three weeks and the plant label put in the box at the final collection. Owing to the fact that the seed pods were not opened until after the collections had been finished, no suspicion of anything unusual was entertained until after any sort of check was no longer possible. A mistake might have been made several times in succession, but this is certainly very improbable. The seeds this year are of the usual type. Three light-blue plants produced light seeds and two dark blues produced no seeds.

If this is not a case of mistake in records it is very difficult to offer any explanation of it. Since plant VIII was a hybrid and the factor for dark-blue flower color is linked with that for dark seeds, plant VIII-27 could have arisen either through a mutation in the recessive factor for coat color or by a cross-over of its dominant

allelomorph, so that dark flowers would then be linked with light seeds. In either event plant VIII-27 would be homozygous for dark flowers. It might have been either homozygous or heterozygous for seed-coat color. In the one case it would yield a progeny with dark-blue flowers and light seed coats. In the other all plants would have dark-blue flowers, but there would be three light seed coats to one dark one. The results, however, are both light-blue and dark-blue flowers.

Pedigree VII is dark blue and has bred true for three seasons. This spring culture VII-3-1 produced a single white plant. It was the only plant from 77 seeds. The parent plant was exceptional in that its seeds were lighter in color than usual.

Discussion.—It is realized that the facts presented in the preceding pages are regrettably incomplete. It is hoped, however, that they are sufficient to interest others in lupines as suitable materials for genetic investigation.

The striking parallelism in the mutations occurring in the two species, *apricus* and *nanus*, is certainly a significant phenomenon. All recent work with both plants and animals proves that in varietal crosses homologous chromosomes are freely interchangeable and that allelomorphic factors occupy identical loci in their respective chromosomes. The work with multiple allelomorphic systems clearly indicates that a particular factor may undergo a number of different changes. In *L. apicus* the evidence at hand likewise indicates that the factors producing striped white and light blue respectively are each allelomorphic to that for dark-blue flower color. Whether they are allelomorphic to one another remains to be shown, though that would be a probable supposition.

From the data presented in this paper it can not be said whether flower color and seed-coat color are both due to the same factor or to linked factors. The latter is, however, indicated by two facts. In the first place we already know more heritable patterns of coat color than there are flower colors associated with them. At least

three seed patterns are found in association with dark-blue flowers in different pedigrees. In the second place there is the case of the dark-blue plant, VIII-27, which produced seeds with the light color characteristic of light blues.

In the great majority of mutations described up to the present time the new character is recessive to the normal one (1). They are apparently due to the loss or inhibition of a previously existing factor. It is interesting to note that the two factors for light blue and striped white here reported are both dominant, the former completely and the latter incompletely so. Furthermore, many of the other variations observed are in the nature of additions. Yellow color on the front of the banner and orange lateral spots, although their heredity is not yet known, are certainly to be considered as in the nature of additions.

The mutations in *Lupinus* represent three categories (11). In the whites a positive character has been lost. In the light blues and striped whites a character has been replaced by another. This would naturally be supposed to be due to some alteration of the factor governing the somatic character. In the case of the orange spots and lemon-yellow fronts one is led to suppose the addition of a new factor, especially so in view of the fact that they are not constantly associated with one another or with white flowers. White flowers may occur without either lemon fronts or orange spots or with both or with one and not the other.

These three categories of characters naturally suggest that there are also three sorts of factorial bases for them. Loss of a character appears readily explicable either on the assumption of an actual loss of the factor in the chromosome or of its becoming latent. Multiple allelomorphic factors seem to be located according to the work of Morgan and his associates (8) at identically the same loci in their respective chromosomes and must therefore be thought of as different changes of the same factor.

Additional characters when inseparably linked with an old one might be due to a change in the original factor, but when not linked, or so loosely so as not to maintain a constant association, they would have to be considered as due to a new factor. A new factor might originate by the subdivision of an old one and the subsequent differentiation of one part. In this case as well as in the case of the actual loss of a gene, homologous chromosomes of the hybrid between the new and old form would present the situation originally conceived in the Presence and Absence hypothesis (2) of an actual gene paired with its absence. In the other cases of modified factors this would not be true.

Summary.—1. The genus *Lupinus* presents an assemblage of closely related and difficultly separable species.

2. The present paper reports some results of a 6-year field and garden study of *L. apicus vallicola*, *L. piper-smithii*, and *L. nanus*.

3. The variations described concern the form and color of the flower, the shape and size of the pod, and the color and markings of the seeds.

4. Dark-blue and pink-flowered races breed true.

5. Striped-white flowered races are heterozygous for a single factor, which in the homozygous condition produces white flowers.

6. Light-blue flowers are due to a single dominant factor, indistinguishable in the homozygous and heterozygous condition.

7. Dark seed coats are linked with dark-blue flower color, but probably due to separate factors.

8. The factors for light-blue and striped-white flowers are both allelomorphic to that for dark-blue and not improbably constitute a system of multiple allelomorphs.

9. Mutations are frequent, some are already known to be dominant, and others appear to be in the nature of additions of new characters and factors and so progressive in the sense of de Vries.

10. On account of the frequency of dominant and progressive mutations and notwithstanding the difficulties of seed germination this genus merits the attention of geneticists.

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